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TITLE: Genome-Wide Analysis of Germline Cnps and Snps in Prostate Cancer

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14. ABSTRACT
Prostate cancer (PCa) is the leading cancer among men in the United States, and is a disease with strong genetic susceptibility. The genetic susceptibility is due to the inheritance of altered germline DNA sequences, either in the form of point mutations such as single nucleotide polymorphisms (SNPs), or deletions/gains of a string of nucleotides such as copy number polymorphisms (CNPs). Most current genetic studies focus only on the role of SNPs in genetic susceptibility. In contrast, few studies have explored the role of deletions/gains in cancer predisposition, due to limited methods. In fact, germline deletions/gains are common in the human genome and may have a significant impact on gene products because they can involve an entire gene or a significant portion of a gene. They may play a more important role in hereditary PCa (HPC), a type of PCa that is likely due to germline changes in major genes. We have achieved great progress in the first year and are on track for the next Aim.

15. SUBJECT TERMS
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INTRODUCTION

Prostate cancer (PCa) is the leading cancer among men in the United States, and is a disease with strong genetic susceptibility. The genetic susceptibility is due to the inheritance of altered germline DNA sequences, either in the form of point mutations such as single nucleotide polymorphisms (SNPs), or deletions/gains of a string of nucleotides such as copy number polymorphisms (CNPs). Most current genetic studies focus only on the role of SNPs in genetic susceptibility. In contrast, few studies have explored the role of deletions/gains in cancer predisposition, due to limited methods. In fact, germline deletions/gains are common in the human genome and may have a significant impact on gene products because they can involve an entire gene or a significant portion of a gene. They may play a more important role in hereditary PCa (HPC), a type of PCa that is likely due to germline changes in major genes.

In this DOD funded proposal, we propose to 1) identify germline CNPs in the genome among high-risk PCa patients using the Affymetrix 500K SNP mapping panel; and 2) test for their associations with PCa risk using family-based association tests.

BODY

Approved Statement of Work: To identify germline CNPs in the genome among high-risk PCa patients using the Affymetrix 500K SNP mapping panel.

1) **(Months 1-4)** Regulatory review and approval process for human studies.
2) **(Months 5-12)** Genotype ~500K SNPs among two affected members from each of the 206 HPC families ascertained at Johns Hopkins Hospital using the Affymetrix 500K SNP mapping panel. We anticipate an average call rate > 95% using the default mapping algorithm setting. By applying methodology recommended by Affymetrix for improving the call rate of heterozygotes, we expect to achieve an average call rate >98%.

Summary report

By February 2008, were in the 12th month of this funded project. We have obtained approval from our Institutional Review Board for this study. We have successfully completed genotyping for 316 subjects for hereditary prostate cancer families using Affymetrix 6.0 SNP array. The quality of genotyping for these subjects is excellent. However, the genotyping call rate was below optimal for the remaining 96 subjects. We are in the process of repeating the assays. Therefore, we are on target with the approved Statement of Work.

Detailed report

Genotyping platform. Initially, we proposed to genotype two affected members in each of the 206 hereditary prostate cancer families using the Affymetrix 500K SNP array. However, we have upgraded our system as an improved Affymetrix SNP array (6.0) became available last year. We believe this platform will help us to accomplish our aims more efficiently. This panel contains more than 906,600 SNPs and more than 946,000 probes for the detection of copy number variation. Among these CNV probes, 202,000 of them target 5,677 known regions of copy number variation from the Toronto Database of Genomic Variants. All SNPs on the Array 6.0 Panel went through a more rigorous screening and validation process than the earlier generations of SNP arrays. The median inter-probe distance taken over all 1.8 million probes is less than 700 bases. Therefore we used the Affymetrix SNP array (6.0) for this analysis. Additional cost associated with the new

version of Affymetrix SNP array is being absorbed through support from our institutional, as proposed in our initial grant application.

Study population. DNA samples of the 206 HPC families from Johns Hopkins Hospital are being analyzed. These families were ascertained from three resources; through referrals generated in response to a letter by Dr. Patrick Walsh to 8,000 urologists throughout the country, from family history records of the patient population seen at Johns Hopkins Hospital for treatment of prostate cancer, and from the respondents to articles published in a variety of lay publications describing our prostate cancer family studies.[1-2] Prostate cancer diagnosis was verified by medical records for each affected male included in this study. Each man's age at prostate cancer diagnosis has been confirmed either through medical records or from two other independent sources. The mean age at diagnosis for men included in the study is 64.3 years. The number of families with 3, 4, and 5 or more affected individuals is 28, 50, and 128, respectively. Eighty-two percent of the families are of Caucasian descent and 9.2% are African descent.

Results. We have successfully completed genotyping for 316 subjects for hereditary prostate cancer families using Affymetrix 6.0 SNP array. The quality of the genotyping for these subjects is excellent however; the genotyping call rate was below optimal for the remaining 96 subjects. For this reason, the experiment is currently being repeated.

For the 316 samples with good genotyping quality, we estimated DNA copy numbers at each SNP and identified germline CNPs in the genome using a computer software package, known as DNA-Chip Analyzer (dChip). Based on the estimated DNA copy number of these SNPs, we identified germline CNPs using a set of criteria implemented in our scripts: 1) DNA copy number cutoff values of ≤ 1.5 for deletions and ≥ 2.5 for gains, 2) a homozygous genotype is required when the CNV indicates a deletion, and 3) must involve at least three consecutive SNPs and a minimum physical length of 1 kb.

Using our working criteria, we identified a number of CNVs, with the frequency of deletions similar to that of gains, which is consistent with those reported by Redon et al. [3] and Wong et al.[4]. To reduce the occurrence of false positive CNVs due to mutations or SNPs in DNA probes and measurement errors, we chose 19 detected CNV regions, including 10 deletions and 9 gains for further validation by quantitative PCR or Sequenom; thus far, we have confirmed 9 of these regions. The detailed results are presented in Table 1 for the CNVs confirmed by alternative methods.

Table 1. Germline CNVs detected in 314 HPC patients

		Positions			Frequency	Known	Known
Chr	CNV	Start	End	Length (bp)	Case	CNV	genes
2p24.3	del	14,657,379	14,660,690	3,311	59	yes	no
7p21.2	del	13,363,656	13,364,914	1,258	13	yes	no
8q13.1	del	67,152,424	67,162,924	10,500	6	novel	DNAJC5B
9q21.13	del	76,380,420	76,386,298	5,878	8	novel	no
11q14.1	amp	83,508,907	83,517,920	9,013	10	novel	DLG2
13q33.1	amp	102,078,138	102,080,300	2,162	11	novel	TPP2
15q11,.2	del	21,606,530	21,610,088	3,558	21	yes	no
19p13.2	del	6,926,998	6,939,270	12,272	5	novel	EMR4
21q22.11	amp	31,676,751	31,693,750	16,999	8	novel	TIAM1

Advantages to using Affymetrix SNP 6.0. With a resolution ~4 times that of the 500K SNP array, the SNP 6.0 array enables us to uncover smaller size CNVs in the genome and map the breakpoints of deletions and gains more precisely. More importantly, the higher resolution of the SNP 6.0 array enables us to identify novel CNVs in addition to those already documented in the database of Genomic Variants (http://projects.tcag.ca/variation/) as shown in Fig 1. It is very interesting that both of these two small CNVs are located within the gene CSMD3 on chromosome 8q.

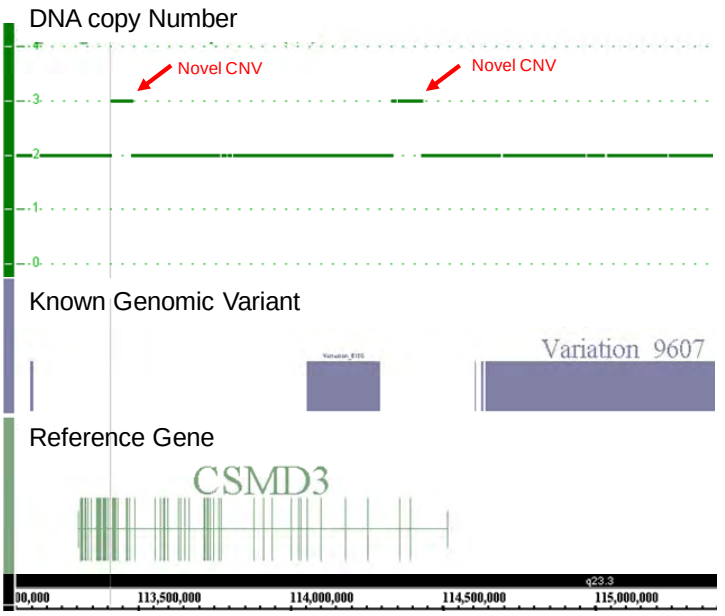

Fig 1. Novel small CNV identified by SNP 6.0 Array.

KEY RESEARCH ACCOMPLISHMENTS

1) We have successfully completed genotyping for 316 subjects of the hereditary prostate cancer families using the Affymetrix 6.0 SNP array.
2) We have identified and confirmed several common germline CNVs in these samples (Table 1).

REPORTABLE OUTCOMES

1) By February 2008, we were in the 12th month of this funded project
2) We have obtained IRB approval for this study
3) We have successfully completed genotyping for 316 subjects for these hereditary prostate cancer families using Affymetrix 6.0 SNP array. The quality of genotyping for these subjects is excellent. However, the genotyping call rate was below optimal for the remaining 96 subjects. For this reason, we are currently in the process of repeating these assays.
4) We have identified and confirmed several common germline CNVs (Table 1).

CONCLUSION

1) We have made great progress in achieving the goals described in the approved Statement of Work.
2) We have identified common germline CNVs in hereditary prostate cancer (HPC) patients.
3) We will test association of these germline CNVs with prostate cancer risk in all HPC family members in the second year.

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